

Plantlets from Paulownia Tissue Culture

by

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Summary

Plantlets were produced from white and firm callus tissue of *Paulownia taiwaniana* Hu & Chang initiated on a modified Murashige and Skoog medium. Illuminated by 400 ft.-c of light for 16 hrs/day at 25°C., leafy shoots were initiated followed by roots at a later stage. Whole plantlets were then isolated from callus. Plantlets after transplanting into the soil survived.

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Introduction

There have been many successful cases in the induction of complete plants from tissue and cell culture of herbaceous angiosperms and monocots (Murashige, 1974) (1). However, plantlets from woody tree tissue culture have been reported only in very limited cases. According to Winton (1974) (2) plantlets have been produced successfully by tissue culture method in the following few trees; *Eucalyptus citriodora* (3), *Ephedra foliata* (4), *Zamia floridana* (5), *Pinus palustris*, *Pinus elliottii*, *Pinus rigida* (6), *Populus tremuloides* (triploid, tetraploid and diploid plants), *Populus alba* (7, 8, 9), *Ulmus glabra* (10), *Ilex aquifolium* (12), *Eucalyptus obliqua*, *Eucalyptus viminalis* (12), *Broussonetia kaziniokii* (13), *Populus deltoides*, *Populus nigra* (14, 15), *Citrus sinensis* (16, 17, 18, 19), *Citrus aurantiifolia* (20), *Citrus maxima* (21), *Populus euramericana* cv. *robusta*, *Manihot esculenta* (22).

This paper reports * the success of organ initiation from *Paulownia taiwaniana* stem callus into complete plantlets and the transplanting of these plantlets in open air.

The genus *Paulownia* is a native of East Asia and has been introduced to Europe and North America for commercial purposes. It is a kind of lumber tree renowned for its light weight combined with strength. It has been used extensively in industries for making chest, guitar and other types of musical instruments. It is also useful for building small boats and as military aircraft plywood.

* A preliminary note under the same title has been published in Chinese in the Taiwan Forestry Research Institute Bulletin No. 286 in December 1976.

Materials and Methods

The physiological conditions required for callus production and organ initiation into plantlets varied somewhat with the age of the explants employed. Generally, the younger the explants, the easier the organ initiation. Two types of *Paulownia taiwaniana* (23) stems, one from seedlings and the other from saplings of approximately one year old were used to initiate callus culture.

1. Seedling stem callus cultures: *Paulownia* seeds were sterilized in 10% w/v calcium hypochloride and sown aseptically on agar medium containing only inorganic salts of Murashige and Skoog medium 1964 (24). Toole and his associates in 1958 (25) found *Paulownia* seeds required more light energy for germination than seeds like lettuce. Thus, for the germination of *Paulownia* seeds, the cultures were exposed to 400 ft-c of light for 16 hours each day. Four to six weeks were required to initiate germination and likewise for the seedling stem to grow to a length of 2 cm with a diameter of 0.05 cm. The seedling stem was very slender and it was very difficult to separate the nodes from the internodes. Subsequently two-thirds of the length from the apex of these slender stems were cut off to induce straighter and stronger new shoots to grow out from the seedling stumps. It took only two to three weeks for a new shoot to grow to a stem of 8 to 10 cm in length and 0.2 cm in diameter. Nodes and internodes of the stem could be clearly distinguished. The seedling stem was excised into nodal and internodal segments of 0.2 to 0.3 cm in length. Nodal and internodal segments were planted separately on 5 different media (Table I). All cultures were grown at 25°C in darkness. Temperature has been found to be an important factor. If the temperature was higher than 28°C degree, the callus culture turned brown.

Table I. Nutrient Medium Composition for Formation of Callus from *Paulownia* (all values expressed as milligrammes per liter).

Constituent	A	B	C	D	E
NH ₄ NO ₃	1650	1650	1650	1650	825
KNO ₃	1900	1900	1900	1900	950
MgSO ₄ 7H ₂ O	370	370	370	370	185
CaCl ₂ 2H ₂ O	440	440	440	440	220
KH ₂ PO ₄	170	170	170	170	85
FeSO ₄ 7H ₂ O	27.84	27.84	27.84	27.84	13.92
Na ₂ EDTA	37.24	37.24	37.24	37.24	18.62
MnSO ₄ ·H ₂ O	16.9	16.9	16.9	16.9	8.45
ZnSO ₄ ·7H ₂ O	8.6	8.6	8.6	8.6	4.3
H ₃ BO ₃	6.2	6.2	6.2	6.2	3.1
KI	0.83	0.83	0.83	0.83	0.415
CoCl 6H ₂ O	0.025	0.025	0.025	0.025	0.0125
CuSO ₄ ·5H ₂ O	0.025	0.025	0.025	0.025	0.0125
Na ₂ MoO ₄ ·2H ₂ O	0.25	0.25	0.25	0.25	0.125
Thiamine, HCl	0.4	0.4	0.4	0.4	0.4
myo-Inositol	100	100	100	100	100
Indole-3-acetic acid	—	—	—	—	5
2,4-D	3.0	0.3	3.0	0.3	—
Kinetin	0.03	0.1	0.03	0.1	2.5
Casein hydrolysate	—	—	3000	3000	—
Coconut Milk	—	—	—	—	15%
Sucrose	3000	3000	3000	3000	3000

2. Sapling stem cultures: Five one year old *Paulownia* saplings of height two-feet tall were grown in pots in the open. Stem segments of 0.3 to 0.5 cm in length and 0.5 to 0.8 in diameter were excised from both green hairy young shoots and old brown lignified stem and planted on 5 different media (Table I). The cultures were incubated in darkness until callus was produced. The callus was then cut into four to six pieces and transferred to 11 different media E, F, G, H, I, F', G', H', I', J, K (Table II) for organ initiation. Medium E, found to produce white and firm callus was chosen as the medium for producing stock callus for organogenesis studies. Thus some of the callus was subcultured in medium E and incubated in darkness for stock callus. All callus tissues for organogenesis studies were incubated under 400 ft-c light for 16 hours per day at 25°C in various media. Eleven media, E, F, G, H, I, F', G', H', I', J, and K were used in organ initiation studies (Table II). All the callus used in these studies were from brown lignified internodal stem. Media E, F, G, H, I are Murashige and Skoog medium with varying concentrations of kinetin and Auxin. Media F', G', H' and I' are the same as F, G, H, I respectively except that there is a higher concentration of ammonium nitrate (2000 mg/l instead of 1650 mg/l). Media J and K are Winton's (26). *Aspen* medium with zero and 0.15 mg/l 6-amino-benzyladenine respectively. The callus tissues planted in various media were all kept under 400 ft-c light for 16 hours/day at 25°C.

Table II. Nutrient Medium Composition for Formation of Plantlets from *Paulownia* Callus (all values expressed as milligrammes per liter)

Constituents	F	G	H	I	F'	G'	H'	I'	J	K
Inorganic salts	MS	MS	MS	MS	MS ¹	MS ¹	MS ¹	MS ¹	W ²	W ²
Organic constituents										
Thiamine, HCl	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.1	0.1
myo-Inositol	100	100	100	100	100	100	100	100	100	100
Nicotinic acid	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Glycine	2	2	2	2	2	2	2	2	—	—
Pyridoxine, HCl	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.1	0.1
Adenine sulfate (dihydrate)	40	80	40	40	40	40	40	40	—	—
Kinetin	1	0.6	2	6	1	0.6	2	6	1	0
6-Benzyladenine	—	—	—	—	—	—	—	—	—	0.15
Indole-3-acetic acid	0.1	0.2	2	2	0.1	0.2	2	2	—	—
Sucrose	3000	3000	3000	3000	3000	3000	3000	3000	2000	2000
Coconut Milk	15%	15%	15%	15%	15%	15%	15%	15%	—	—
2, 4-D	—	—	—	—	—	—	—	—	0.04	—

1 NH_4NO_3 2000 mg per liter, the rest of the composition as Murashige & Skoog Medium (1962)

2 Same as Winton Medium (1970)

Results

Stem segments excised from green hairy young shoots of *Paulownia* harboured all kinds of contaminants in the hairs and were very sensitive to surface sterilization with calcium hypochloride. They had no chance to form callus for they were killed by either contamination or sterilization procedures. On the other hand the brown lignified stem can resist sterilization procedures. The outside layers were easily peeled off before segmenting into lengths of 0.3 to 0.5 mm and planting on the media. Thus more than 90% stem segments in culture formed callus after four to six weeks' growth in darkness.

Fine media (Table I) were used to produce *Paulownia* stem callus. On medium A, callus was produced in the first week but appeared to be a very loose soft structure of brown color. Media B and C produced firm callus of mixed white and brown color. Medium D failed to produce callus. Medium E produced white and firm callus. Thus callus from medium E was chosen for organogenesis studies. The time required to form callus from different stem sections of *Paulownia* on Medium E is as shown in Table III.

Table III. Callus Formation from Stem Segments

	% formed callus	Time required to form callus
Seedling internode	100%	2-3 weeks
Seedling node	100%	2-3 weeks
lignified brown stem node	100%	4-6 weeks

It has been found that in brown lignified stem sections, culture callus grew from the cambium and phloem regions, and thus looked like a doughnut in shape. When the callus was cut into four to six pieces and subcultured on medium E under the same conditions each piece grew into a new lumpy callus of 2 cm diameter in three weeks (Fig. 1).

After two to three weeks, the callus planted in media F, G, H and I enlarged quickly and turned bright green in colour. Though the callus appeared as if it would sprout any time yet it turned brown and withered after a couple of months with no formation of shoots at all. The callus in media F', G', H' and I' showed a pale green colour and no shoots came out after two months. The callus in media J and K grew slowly, turned into a brown colour and finally died. Surprisingly, the callus in medium E showed green spots and started to sprout (Fig. 2) after several weeks exposure to 400 ft-c light for 16 hours per day at 25°C. The time required for shoots to sprout from callus tissue from various stem sections is shown in Table IV.

Table IV — Shoot Formation from Callus

	% Sprouting	Time required to sprout
Seedling internode	50-60 %	3- 5 weeks
Seedling node	95-100%	2- 3 weeks
Callus from brown lignified stem-internode and subcultured several times	20-30 %	6-10 weeks

Two or three weeks after the sprouting, roots started to grow out and whole plantlets were isolated (Fig. 3). The plantlets after transplanting into the soil survived (Fig. 4).

Callus from seedling node has almost 100% chance to sprout shoot compared to callus from stem internode section. Possibly because node have pre-existing axillary buds (meristem). Cells from meristem tissue usually are more active than cells from other tissues and are always more easy to form adventitious bud. Callus from the lignified internode stem showed a lower percentage of sprouting than callus from seedling internode. There are three possible reasons to explain this. Firstly, all the callus from lignified stem segments for organ initiation studies have been subcultured several times. The same phenomenon has been reported in carrot tissue (27). Secondly, the more subculturing done, the lesser the origin cambium tissue left. Cambium is a kind of meristem tissue and may form adventitious bud. Thirdly, the brown lignified tissue is older than tissues of the seedling stem. The younger the tissue, the easier it is to form adventitious bud.

Results of organ initiation in the 10 media indicate that tests on *Paulownia* requires a higher concentration of auxin than kinetin. Adverse results were shown in the media F, G, H, I, F', G', H' and I' where there was less kinetin than auxin. The callus turned green but no shoots were formed whereas in medium E with the concentration of auxin doubled that of kinetin the callus sprouted. Yet, medium E had only half the strength of inorganic salts of the other eight media. Whether half strength instead of full strength of Murashige and Skoog inorganic salts is of critical importance for organogenesis or not requires further research.

It is observed that the seedling with the top cut off produced new shoots which grew faster and straighter. This is also true for *Paulownia* sapling in the forest. There must be some hormone stimulation for adventitious shoot formation following the removal of the stem apex. However, some plantlets isolated from callus grew even faster than adventitious shoots from the seedling stumps in flask culture. This may be due to the effects of auxin and kinetin which were present in the media used for organ initiation. It is also possible that quick growing plantlets isolated from callus are polyploids (28). Further research on the chromosomes of the plantlets can reveal whether the faster rate of growth of the plantlets isolated from callus is due to polyploidy or not. Plantlets from tissue culture method of some other woody plant has been reported to be difficult to survive in the open on soil because of the defective root system. They dehydrated and died after transplanting in the soil. However, plantlets of *Paulownia* produced in this experiment after transplanting into the soil survived (Fig. 4).

It has been found that medium E is very good for callus formation. Medium E has induced the formation of white and firm callus from:— rice anther in anther culture, chrysanthemum stem, rose stem, dahlia underground stem and some other plants. In the case of *Begonia*, new plantlets have been found on segments of flower stalks in medium E under direct light.

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